or transform one or more host cell strains, expressing said DNA in one or more of said host cell strains, and analysing expression products from said host cells for solubility.

45. (Previously presented) A method of producing a soluble bioactive domain of a protein of interest comprising the steps:

(a) analysing DNA coding for the protein of interest to identify one or more candidate soluble domains,

(b) providing oligonucleotide primers to amplify DNA encoding each domain,

(c) amplifying said DNA using, in parallel, a standard PCR programme using a predicted annealing temperature for the primers; (ii) a standard PCR programme using a temperature in the range 48 to 52°C, preferably 50°C, as the temperature for annealing; and (iii) a touchdown PCR programme, where the annealing temperature starts at a temperature in the range 62 to 67°C, and then gradually decreases to a temperature in the range 48 to 52°C, over the subsequent cycles,

(d) cloning amplified DNA from step (b) into a plurality of different expression vectors, at least one of which vectors is capable of encoding a fusion protein with a solubility enhancing tag comprising a SNUT tag,

(e) optionally screening clones for correct orientation of DNA,

(f) using each of the vectors of step (d) into which the DNA has been cloned to each transfect or transform a plurality of different host cell strains,

(g) expressing said DNA in one or more of said host cell strains, and

(h) analysing expression products from said host cells for solubility.

Remarks

Claims 1-26, 31-40, 44 and 45 are pending in the application. Claims 33, 34, 37, 38 and 40 have been amended to delete reference to Figure 8 in favor of the appropriate sequence identifier.

The amendment to claims 34, 38 and 40 replaces reference to "amino acids 26-171 of the SrtA sequence shown in Figure 8" with "amino acids 13-157 of SEQ ID NO:5". Support for this amendment is summarized as follows. First, the numbering of the amino acids in the description and claims referred to the numbering in the Genbank sequence for SrtA, *i.e.*, reference to "amino acids 26 -171 of the SrtA sequence shown in Figure 8" does not mean the 26th to 171st amino acids recited in Figure 8, but the amino acids corresponding to the SrtA Genbank sequence at residues 26 to 171. Second, the reference to residue 171 of the SrtA sequence of Figure 8a in the application as filed was made in error. This residue, which corresponds to residue 158 of Figure 8, when the first amino acid of Figure 8 is taken as residue 1, is the amino acid residue corresponding to the nucleotide triplet GGA which together with the subsequent triplet TCC is, as described by the examples of the present application, a BamH1 restriction site and not a SrtA amino acid. The basis for the amendment to claims 34, 38 and 40 is described in greater detail, as follows.

Support for the amino acid numbering referring to Genbank accession no: AF162687

In the description and the claims as originally filed, reference is made to the nucleic acid and amino acid sequences shown in Figure 8. Particular reference is made to SrtA gene products comprising amino acids 26-171 of the SrtA sequence shown in Figure 8 (see, for example, page 9, lines 1-3, and each of claims 30, 34, 38, and 43 as originally filed). However, the reference to "amino acids 26 -171 of the SrtA sequence shown in Figure 8" does not mean the 26th to 171st amino acids recited in that figure, but was intended to refer to residues 26 -171 of the Genbank SrtA sequence. Thus, the amino acid residue numbers referred to on page 9, lines 1-3 correspond to the numbering of the amino acid residues of the Genbank sequence for SrtA (AF162687). Reference to this sequence as being the Genbank sequence for SrtA is made in the application as filed, page 23, lines 8-15. In the amino acid sequence shown in Figure 8a (SEQ

ID NO:5), the amino acid residue corresponding to the residue 26 of the Genbank sequence for SrtA (AF162687) is the 13th amino acid.

That the amino acid sequence numbering corresponds to the numbering system as used in the Genbank reference will be clear to the skilled person based on the teaching of the application as filed. From the examples describing the construction of the SNUT vector shown in Figure 8 (Examples 7, 8 and 9), it is clear that the region of the amino acid sequence of Figure 8a that is a SrtA fragment corresponds to amino acid residues 13-157 of that sequence. In particular, Example 7 describes the construction of the SNUT expression tag, as illustrated in Figure 8. The first sentence of this example (page 40. lines 21-23) teaches that it was decided to amplify the region of amino acids 26-171 of the SrtA sequence. Example 7 describes the primers used and proceeds to teach that the amplified product was digested with BglI and BamHI and ligated into the pQE30 vector to form the pSNUT vector (page 40, lines 27-30), with the example on page 41 lines 9-12 describing that the sequence of the multiple cloning region of the resultant vector (the pSNUT vector) is shown in Figure 8. The nucleotide sequence corresponding to the BamH1 site is well-known in the art as GGATCC, with cutting occurring between the two guanines. In the sequence of Figure 8a, the nucleotide sequence corresponding to the BamHI site is found at nucleotide residues 472-477 and thus the C terminal of the SrtA amino acid sequence will be at residue 157 of Figure 8a, when taking the first amino acid residue recited in Figure 8a as residue 1.

Given that the first sentence of Example 7 describes the region of the SrtA sequence to be amplified for subsequent insertion into the pQE30 vector to generate the pSNUT vector, it is clearly implied that the amino acid numbering is that of the known SrtA sequence (instead of using the numbering of amino acid sequence corresponding to the as yet unconstructed vector). The known SrtA sequence is referenced in the following paragraph as Genbank AF162687 (page 40, lines 12-15). Based on this, it would be clear to the skilled person that the reference to "amino acid residues 26-171 of the SrtA sequence of Figure 8" throughout the specification should be taken to refer to the numbering system as used in the known SrtA sequence, *i.e.*, as in Genbank AF162687.

Accordingly, it is clear that in the description and claims as originally filed, the reference to amino acids 26 -171 of the SrtA sequence shown in Figure 8 does not mean the 26th to 171st amino acids recited in that figure.

Support for the SrtA sequence being amino acids 13-157 of SEQ ID NO:5, and not amino acids 13-158 of SEQ ID NO:5

The reference to residue 171 of the SrtA sequence of Figure 8a in the application as filed was made in error. This residue, which corresponds to residue 158 of Figure 8, when the first amino acid of Figure 8 is taken as residue 1, is the amino acid residue corresponding to the nucleotide triplet GGA which together with the subsequent triplet TCC is, as described by the examples of the present application, a BamH1 restriction site. As noted above, Example 7 teaches that the sequence encoding the SrtA fragment was ligated into the pQE30 vector at a BamH1 restriction site to form the pSNUT vector (page 40, lines 27-30). The skilled person will clearly recognize the BamH1 site in the vector sequence of Figure 8. In Example 8, the cloning of target inserts into the pSNUT vector is described and clarifies that such inserts are cloned into the BamH1 site (page 40, lines 24-28). As the skilled person would be aware, the BamH1 nucleotide restriction site comprises nucleotides GGATCC. Thus, it would be clear to the skilled person that residue 158 of Figure 8 (SEQ ID NO:5) was not a residue of the SrtA sequence, and that the amino acids shown in Figure 8 that are SrtA amino acids are amino acids 13-157, when the first amino acid shown in Figure 8 is numbered as amino acid 1.

In conclusion, the specification supports replacement of "amino acids 26-171 of the SrtA sequence shown in Figure 8" in claims 34, 38 and 40 with "amino acids 13-157 of SEQ ID NO:5".

Respectfully submitted,

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